DuPont Qualicon (formerly DuPont FQMS Group)

Testing Food-Borne Bacteria Using DNA Analysis

As of 1994, food contamination was causing 10 million illnesses and nearly 4,000 deaths each year in the United States. Testing food samples for bacteria was costly and time consuming, requiring repetitive manual processes. Researchers at DuPont FQMS Group believed they could test food samples for contamination by using automated DNA analyses, a process that the health industry was using to analyze patient blood and tissue samples for disease. The company applied to the Advanced Technology Program (ATP) for cost-shared funding under a 1994 focused program, "Tools for DNA Diagnostics." The company's proposed project had high technical risk, because it required integrating many technologies, including miniaturization, micro-separation, contamination prevention, and computer analyses.

ATP awarded funding for a three-year research project beginning in 1995. DuPont FQMS (later called DuPont Qualicon) developed a functioning prototype to analyze DNA. The prototype used microfluidics (tiny amounts of fluid moving on a micro-scale), which reduced analysis time from 3 hours to 30 minutes, a sixfold improvement. The company received a patent for this work. Unfortunately, using a smaller sample increased the sample preparation time, which negated the time saved by the DNA analysis system. As a result, DuPont suspended further development of the technology until sample preparation methods were improved. No commercialization has resulted to date, and DuPont is now pursuing alternative bacteria-testing methods.

COMPOSITE PERFORMANCE SCORE
(based on a four star rating)

No Stars

Research and data for Status Report 94-05-0033 were collected during June – July 2004.

Food Sample Testing Was Slow and Costly

In the 1990s, DuPont FQMS Group was researching improved methods for testing bacterial contamination in foods. Although the company looked to the medical products industry for improved methods, testing for food-borne bacteria was more complicated than testing blood and tissue samples for disease. Bacteria such as *Salmonella* and *E. coli* involved a labor-intensive manual testing process that required skilled operators and provided low throughput (production). Moreover, samples required six days or more to generate results.

In a well-publicized 1993 case of food poisoning, four children died and hundreds of people became ill from eating hamburgers at a fast food chain that were infected with *E. coli*. As of 1994, as many as 4,000 Americans died and 10 million became ill each year from eating bacteria-infected food.[1] The annual number of premature deaths caused by food-borne pathogens exceeded deaths from accidental fire (3,300) and drowning (3,300).[2] More than 200 known diseases are transmitted through viruses, bacteria, parasites, toxins, and metals in the food supply.[3] Symptoms of food-borne illness range from mild gastroenteritis to life-threatening neurological problems, and liver and kidney failures. Infections in the U.S. population from the five major bacterial pathogens (*E. coli* O157:H7, *E. coli* non-O157 STEC, *Campylobacter*, *Listeria monocytogenes*, and *Salmonella*) cost $6.9 billion annually, according to a USDA Economic Research Service report in 2000. That estimate includes medical costs, productivity

[1] *Wall Street Journal*, CCXXVIII, no. 10, July 15, 1996.

[2] P. D. Frenzen, "Deaths Due to Unknown Foodborne Agents." *Emerging Infectious Disease* September 2004.

[3] Bryan, F.L. "Diseases Transmitted by Foods." Atlanta: Centers for Disease Control, 1982; P. S. Mead, L. Slutsker, V. Dietz, et al. "Food-Related Illness and Death in the United States," *Emerging Infectious Disease* 1999, 5:607-25.

losses from missed work, and the estimated value of premature death.[4] As a result of the increasing problem of food contamination, consumers began to demand increased testing for bacterial contamination in food, and public concern has remained steady since the mid-1990s.

The U.S. food market was substantial. For example, in the mid-1990s, the annual U.S. market for meat alone was $65.2 billion and was $28.5 billion for chickens and eggs. Although consumer demand for food safety testing was rising sharply, the technology used to test food samples for bacterial contamination had not improved significantly. The primary testing process, called amplification-based DNA technology, consisted of the following costly and time-consuming steps:

- First, the sample, such as 65 grams of hamburger meat combined with 650 milliliters of enrichment broth, was prepared. Preparing a small representative sample took 24 to 48 hours.
- After a representative sample was ready, technicians amplified the representative DNA sequences (making many copies), using a process called polymerase chain reaction (PCR). PCR can generate 100 billion copies of the DNA in a few hours.
- Finally, the DNA sequences were analyzed and reported. Existing testing methods to analyze the DNA relied on fluorescent detection, which pairs RNA molecules with genes that express them (called hybridization capture). Technicians measured the fluorescent signal at specific probe locations to determine the presence or absence of signals for specific bacteria.

Another method to analyze the DNA sequences was capillary electrophoretic separation. The DNA samples were placed in a capillary (a long, narrow tube) containing a gel medium called agar or agarose. An electric current flowed through the tube, forcing the DNA molecules to pass through the medium at different rates, depending on their size. Smaller fragments moved faster; larger ones moved slower. As a result, the DNA molecules separated into bands, so they could be identified by fragment size.

In 1994, commercial use of amplification-based DNA technology was limited due to cost. The only product approved for routine commercial use at the time was a kit for *Chlamydia* testing. Existing automated systems for detecting contaminants were based solely on some means of hybridization capture, or base pairing. They required skilled operators and had high material costs. As a result, DNA analysis systems' full potential was not being realized.

DuPont Proposes to Automate Sample Analysis Using Capillary Electrophoresis

Researchers in the medical field were relying increasingly on automated DNA analysis for disease testing of blood and tissue samples. Researchers at DuPont FQMS Group wanted to use similar automated techniques to test food samples for bacteria. DuPont had already invested $50 million to develop DNA-based diagnostic systems for microbial testing. The DuPont FQMS Group proposed to develop cost-effective, automated DNA diagnostics that would require minimal technician skills or specialized laboratory facilities. DuPont researchers believed that detection by capillary electrophoresis (analysis by fragment size) would be more versatile than hybridization (RNA base pairing). Capillary electrophoretic analysis would provide opportunities for automation, because it could detect the presence or absence of a specific DNA fragment by its length.

As of 1994, as many as 4,000 Americans died and 10 million became ill each year from eating bacteria-infected food.

DuPont planned to make a simple, automated system, affordable enough to be used in a wide range of laboratories, to support food processing and packaging plants. Their intent was to adapt the existing technology for routine use, so that technicians could operate it without receiving extensive training. Miniaturizing and then integrating the various processes proved to be technically risky; miniaturizing and integrating heightened the need for contamination prevention measures at the micro scale. Because the new system was to handle a wide range of food product samples, the design would be more complex than systems being

developed for the medical industry, which tested only tissue, blood, and urine samples.

In 1994, DuPont submitted a proposal to develop a functioning prototype food sampling system to ATP's "Tools for DNA Diagnostics" focused competition. DuPont's assessment showed that the proposed system would have a significant economic impact on the food processing industry as well as on agriculture, forensics, and medical sectors. If successful, consumers would benefit from improved safety of the food supply. The target markets were all food processors, commercial testing labs, and government regulators. In addition, the agriculture sector could test their crops before incorporating them into the food stream, thus eliminating contamination at the source. Forensic scientists could test more easily for deaths caused by food poisoning. Fewer cases of food contamination could save lives and reduce hospitalizations. Because of the risk associated with miniaturizing, internal funding was not available at DuPont. Therefore, ATP awarded DuPont a three-year, cost-shared award that began in 1995.

DuPont Conducts Parallel Research

Because food bacteria testing was a high priority for DuPont FQMS Group, the company explored multiple avenues simultaneously to maximize its success. In 1996, while the ATP-funded project was ongoing, DuPont released a commercial product for food bacteria testing called BAX, which had been developed through a separate research program. The BAX system screened for pathogenic microorganisms by amplifying specific DNA sequences and detecting the sequences by gel electrophoresis, a multiple-step process that is similar to capillary electrophoresis. DuPont hoped to enhance the benefits expected to result from the BAX product line with the results of this ATP-funded project.

PCR/Capillary Electrophoresis Achieved Some Success

DuPont FQMS researchers developed a functioning automated prototype, which combined PCR (to amplify DNA fragments) with capillary electrophoresis (to separate the fragments by size). In order to prevent contamination, researchers also developed a sealed plastic disposable unit to contain the samples. They were granted one patent for this integrated capillary electrophoresis system. The system delivered reagents to the sample in tablet form (reagent shelf life reached three years, which reduced cost of maintaining and storing reagents). The prototype used microfluidics (tiny amounts of fluid moving on a micro scale), which reduced analysis time from 3 hours to 30 minutes. However, DNA pattern results from sample testing were somewhat inconsistent and needed further development. In 1997 when the ATP-funded project ended, DuPont researchers believed that an integrated, automated system could offer many commercial advantages to the company's existing manual BAX system if the consistency of the pattern results could be improved.

DuPont planned to make a simple, automated system, affordable enough to be used in a wide range of laboratories, to support food processing and packaging plants.

Unfortunately, the miniaturized equipment required smaller samples. Sample preparation time for a typical 50-microliter sample (a microliter is one millionth of a liter) took 24 to 48 hours. However, this preparation time increased when preparing the smaller 1-microliter representative samples. Producing a smaller representative sample led to longer enrichment times or more complicated preparation procedures (for DNA extraction and clean up). As a result, the time saved by the automated system was lost.

DuPont Continues to Develop Bacteria Testing Methods

DuPont FQMS (later renamed DuPont Qualicon) could not justify continuing development of the automated system. Upon project conclusion in December 1997, researchers pledged to continue evaluating and refining the technology in the future. They did apply some of the basic PCR automation knowledge to their existing BAX system, but this project ended in 1998.

Sample preparation time has improved with technology. As of 2004, DuPont Qualicon is able to prepare food samples for DNA testing in about 8 hours (a threefold to sixfold reduction). As miniaturized electronics become more common, it is expected that DuPont will

reevaluate the advances it achieved in microfluidics during this project.

Conclusion

DuPont FQMS Group (later called DuPont Qualicon) believed that food-borne bacteria could be tested more quickly and less expensively by an automated system that combined polymerase chain reaction (PCR) and capillary electrophoresis. The research group sought to miniaturize and automate the testing process to meet strong consumer demand for improvements in the safety of the U.S. food supply. In 1995, ATP awarded cost-shared funding to DuPont to develop an automated system that would reduce testing time to improve food quality for consumers, as well as provide benefits to the agriculture and forensics industries.

DuPont built a functioning prototype that reduced testing time from 3 hours to approximately 30 minutes. The company was awarded one patent based on this technology, but additional steps were required in sample preparation that negated the time saved in analysis. DuPont Qualicon ended the research into this automated system in 1998, but the company did apply some of the automation knowledge gained in this project to its ongoing alternate food-borne pathogen-testing technologies.

PROJECT HIGHLIGHTS
DuPont Qualicon (formerly DuPont FQMS Group)

Project Title: Testing Food-Borne Bacteria Using DNA Analysis (Automated DNA Amplification and Fragment Size Analysis)

Project: To develop an automated, rapid DNA diagnostic system that can determine the presence or absence of specific microbial contamination as a means of quality control in the food industry.

Duration: 1/1/1995–12/31/1997
ATP Number: 94-05-0033

Funding (in thousands):**

ATP Final Cost	$1,966	75%
Participant Final Cost	$ 639	25%
Total	$2,605	

Accomplishments: With ATP funding, the DuPont FQMS Group (later called DuPont Qualicon) conducted a comprehensive study of food-borne bacteria testing. Because of the high risk of this project, the company would not have attempted this research without ATP support. The supporting technologies (such as sample preparation of minute volumes) required to commercialize the automated, miniaturized polymerase chain reaction (PCR) and capillary electrophoresis technology were not sufficiently developed during the project. However, the researchers made several advancements:

- Automated PCR technology
- Reduced analysis time from 3 hours to 30 minutes
- Miniaturized instrumentation and integrated PCR with capillary electrophoresis in one tool (the reduced sample size made sample preparation more complex, which negated the analysis time savings)
- Delivered reagents in the form of tablets, which had a three-year shelf life

DuPont researchers filed for and received one patent from this ATP-funded project:

- "Apparatus for integrated polymerase chain reaction and capillary electrophoresis" (No. 6,372,484: filed January 21, 2000; granted April 16, 2002)

Commercialization Status: DuPont Qualicon does not plan to commercialize automated capillary electrophoresis technology developed under this ATP award.

Outlook: The outlook for DNA testing of food-borne bacteria using an automated PCR/capillary electrophoresis system is poor. However, DuPont Qualicon has been successful in using fluorescence detection methods for its BAX system. Although the company does not plan to develop the proposed automated system, some of the miniaturization and automation knowledge gained during the ATP-funded research may be applied to future projects.

Composite Performance Score: No Stars

Focused Program: Tools for DNA Diagnostics, 1994

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** As of December 9, 1997, large single applicant firms are required to pay 60% of all ATP project costs. Prior to this date, single applicant firms, regardless of size, were required to pay indirect costs.

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